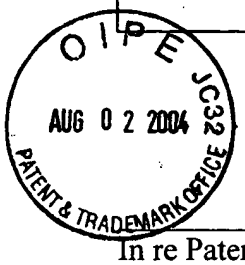
I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: **Mail-Stop** Amendment Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: July 29, 2004 Signature: Nabeela R. McMillian



Docket No.: 27611/35996A
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Rasenick et al.

Application No.: 09/918,230 Group Art Unit: 1651

Filed: July 30, 2001 Confirmation No. 3414

For: MARKER FOR ANTIDEPRESSANT THERAPY AND METHODS RELATED THERETO Examiner: Ralph J. Gitomer

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. MARK M. RASENICK

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Dr. Mark M. Rasenick, do hereby declare and state as follows:

1. I along with Robert J. Donati and Sadamu Toki am an inventor of the invention claimed in the above-referenced application.

2. I familiar with the contents of the above-identified U.S. patent application (hereinafter, the "patent application") and with the official action from the United States Patent and Trademark Office (hereinafter, the "Patent Office") dated March 29, 2004, a copy of which is attached hereto as Exhibit A. A copy of the claims that I understand are pending in the reference application are attached hereto as Exhibit B.

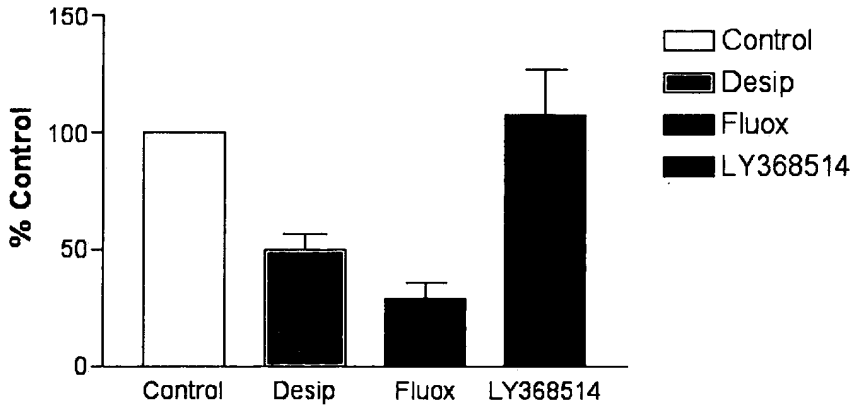
3. I am making this declaration in order to provide facts relating to experiments that I have performed in order to show that the methods claimed in the subject application can be used to distinguish whether a given agent has antidepressant activity.

4. Desipramine and fluoxetine (also known as Prozac™) are antidepressant agents. LY368514 is an inactive fluoxetine analog. LY368514 has a structure nearly identical to fluoxetine, but the CF_3 that is in a para position in fluoxetine is in the ortho position in the LY368514 compound. LY368514 does not block 5HT uptake and does not

have antidepressant properties in behavioral tests (Wong et.al, Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. Life Sci *57*, 411-441, 1995).

5. In order to determine whether there is an effect on the localization of Gsα in C6 cells as a result of treatment with antidepressants, cells were treated with the desipramine or fluoxetine and the data of the localization of Gsα in a TTX-100 insoluble lipid raft rich domain as compared to a more TTX-100 soluble domain were obtained from such treatment. These data were compared to the data observed with a similar treatment using the inactive fluoxetine analog LY368514. More particularly, C6 cells were treated chronically with desipramine, fluoxetine, or the inactive fluoxetine analog, LY368514 (3 days, 10 μM), lysed in buffer containing 1% TTX-100. The detergent insoluble membranes were floated on sucrose density gradients and analyzed by SDS-PAGE and immunoblot for Gsα content. Autoradiographs were compared by densitometry and results were plotted on a graph using Prism Graphpad 4.0.

6. The data from these experiments is depicted in the following figure:



7. The above data show that that antidepressant treatment (i.e., treatment with desipramine or fluoxetine) of C6 cells causes a shift in the localization of Gsα from a TTX-100 insoluble lipid raft rich domain to a more TTX-100 soluble domain. It is noted that

both desipramine and fluoxetine had similar effects in moving Gsα out of cytoskeleton-associated domains (caveolae in this case) and into a more detergent soluble fraction of the membrane.

8. In contrast to the redistribution seen with fluoxetine, the above data show that treatment with LY368514 does not redistribute Gsα into a more detergent soluble fraction of the membrane.

9. In other experiments, I noted that drugs that block neurotransmitter uptake (amphetamine) and tricyclic compounds that are similar in structure to desipramine (chlorpromazine) but that do not have antidepressant properties, also do not have the effect of causing a redistribution of Gsα into a more detergent soluble fraction of the membrane.

10. The above data show that antidepressant treatment of C6 cells causes a shift in the localization of Gsα from a TTX-100 insoluble lipid raft rich domain to a more TTX-100 soluble domain. TTX-100 insoluble lipid raft rich domain is a more strongly hydrophobic domain than a TTX-100 soluble domain. The above data further show that the same experiments performed using agents that are closely structurally-related to the antidepressants tested but that do not have antidepressant activity do not cause this redistribution in the localization of Gsα. Therefore, this effect of antidepressants on the localization of Gsα may be used to distinguish agents that are antidepressants and those that are not.

11. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Date 28 July 2004

Dr. Mark M. Rasenick